Report for 2002TX60B: Fate of a Representative Pharmaceutical in the Environment

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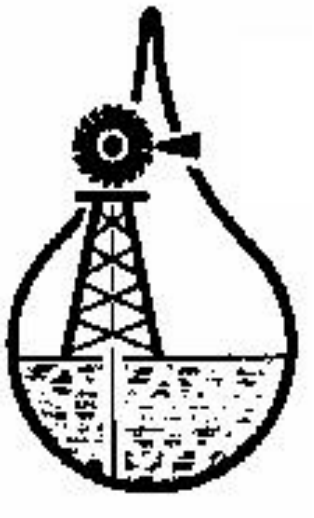

Fate of a representative pharmaceutical in the environment

Final Report

Submitted to

Texas Water Resources Institute

By:

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TEXAS TECH UNIVERSITY

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ABSTRACT

The purpose of this research was to determine the fate of amoxicillin in the City of Lubbock's Water Reclamation Plant and to determine the antibiotic resistance patterns in the plant. Amoxicillin was detected in the influent of the plant during one month of the study, but amoxicillin was not detected at any other plant flow streams. The antibiotic resistance patterns of the LWRP varied monthly; heterotrophic bacteria were resistant to most of the antibiotics investigated during the nine month study.

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INTRODUCTION

As existing potable water supplies are depleted and populations continue to grow in arid and semi-arid areas of the country, including West Texas, the need for complete recycling of wastewater for water distribution may become necessary. Already the dilution factor for wastewater effluent continues to decrease with shorter and shorter intervals between release and reuse. Many municipalities are in fact using treated effluent in their primary water source although it may have spent some time in a natural water course. Historically, the concern with recycled wastewater has been the presence of disease-causing organisms called pathogens. However, a more recent concern of reusing wastewater for consumption is the presence of chemical contaminants, including a new category of compounds: personal care products and pharmaceuticals. Pharmaceuticals, including anti-inflammatories, antibiotics, caffeine, hormones, antidepressants, and others have been observed in various water bodies (Ternes et al., 1998; Heberer et al., 1998; Hirsch et al., 1999; Qiting and Xiheng, 1988).

Antibiotics are one especially troubling class of compounds due to the build-up of resistance in microbial populations. Antibiotics enter the environment from a variety of sources including discharges from domestic wastewater treatment plants and pharmaceutical companies, runoff from animal feeding operations, infiltration from aquaculture activities, leachate from landfills, and leachate from compost made of animal manure containing antibiotics (Figure 1). However, antibiotics are not confined to the natural aquatic environment. Detectable concentrations of antibiotics have been observed in tap water (Herberer et al., 1998; Masters, 2001). The startling fact is that these compounds are passing through water treatment processes and contaminating drinking water supplies. The concentrations of these contaminants typically range from nanogram/liter (ng/L) to microgram/liter (µg/L); the consequences of their presence at these concentrations are unknown. The overall potential for antibiotic removal by biological and physiochemical treatment systems and simultaneous risk of antibiotic resistance development has been relatively unexplored.

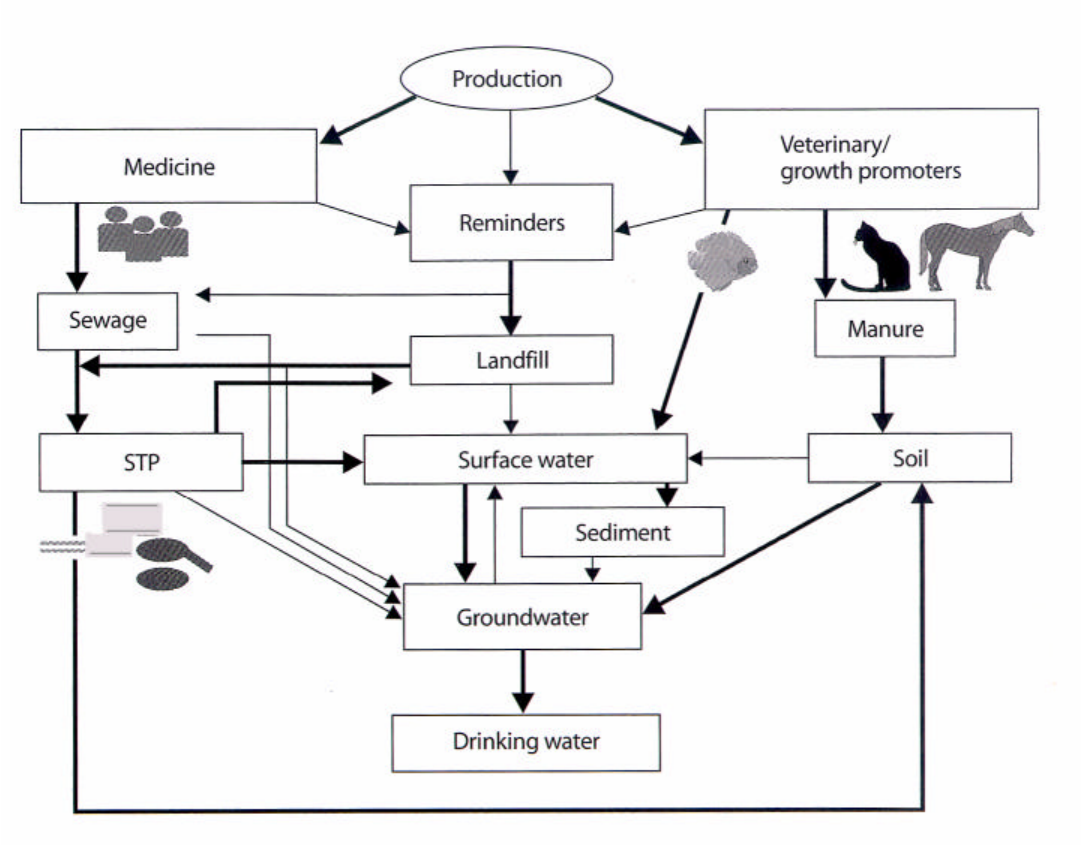

Figure 1. Sources, pathways, and sinks of pharmaceuticals (Kummerer, 2001).

Research has begun to determine the concentrations of antibiotics in the environment, and from this information, the health effects to humans and animals may be estimated by toxicologists. An additional problem that may be created by the presence of antibiotics at low concentrations in the environment is the development of antibiotic resistant bacteria. In recent years, the incidence of antibiotic resistant bacteria has increased and many people believe the increase is due to the use of antibiotics (Walter and Vennes, 1985). The presence of antibiotics can result in selective pressure that favors organisms that possess genes coding for antibiotic resistance. This may pose a serious threat to public health in that more and more infections may no longer be treatable with known antibiotics (Hirsch et al., 1999). In the event that antibiotic resistance is spread from nonpathogenic to pathogenic bacteria, epidemics may result. In fact, bacteria have been observed to transfer their resistance in laboratory settings as well as the natural environment (Kanay, 1983).

The objective of this research was to investigate the effect of a representative pharmaceutical in a biological water reclamation system. The antibiotic evaluated in this study was amoxicillin, which is a semi-synthetic, beta-lactam antibiotic used for a variety of infections. The focus of this particular project is to determine the fate of amoxicillin in the City of Lubbock's Wastewater Reclamation Plant and to determine the antibiotic resistance patterns in the plant.

BACKGROUND

Pharmaceuticals are used in large quantities in human and veterinary medicine or as food additives in animal production (Stan and Heberer, 1997). In animal feeding operations, antibiotics are often prescribed as a preventative measure to keep the animals healthy. The abuse of antibiotics has been rampant since Fleming's discovery of penicillin. Antibiotics were prescribed for the treatment of many illnesses and at doses that may have been inappropriate. There are many forms of antibiotic misuse and abuse. For instance, viral illnesses should not be treated with antibiotics. Also, patients should be educated on compliance issues and the importance of proper use of the antibiotic. Misuse, which includes not completing the prescription, can lead to resistance development (Leiker, 2000a). Preventative measures that may be taken by a clinician to reduce antibiotic resistance development include using the most appropriate spectrum antibiotic for each infection, shortening the duration of antibiotic treatment, knowing local resistance patterns, and limiting antimicrobial prophylaxis if possible (Leiker, 2000a).

Due to the overuse of antibiotics, bacteria have developed resistances to antibiotics. There are three main modes of antibiotic resistance that generally render the antibiotic ineffective, but not all bacteria use the same resistance mechanisms. The first mechanism prevents the antibiotic from binding with and entering the organism, which has been observed in some *P. aeruginosa* (Leiker, 2000b); this form of resistance is related to Multi-Drug Efflux. Other examples are *Steptococcus pnuemoniae* and *Group A Streptococci* penicillin-resistant mutants that have been isolated in the laboratory due to immense and common selective pressure; these mutants contain altered penicillin-binding proteins (Tomasz and Munoz, 1995). The second type of resistance mechanism is the production of an enzyme that inactivates the antibiotic. The classic example of this resistance mechanism is the production of beta-lactamase enzymes in *H. influenze* and *M. catarrhalis*, which destroys the beta-lactam ring of the beta-lactam antibiotic. There are many different enzymes produced by bacteria that are capable of degrading the beta-lactam ring. Fortunately for bacteria, this type of resistance may be spread to other bacteria through a process called "transference" (Leiker, 2000b). The last form of bacterial resistance is the change in the internal binding site of the antibiotic. For

example, the site to which the antibiotic binds has been altered so that the antibiotic may no longer bind, which makes the bacteria are resistant to the antibiotic. This process has been observed in penicillin-resistant *S. pneumoniae*.

Antibiotic resistance may spread using various mechanisms, including conjugation, transduction, and transformation. In conjugation, DNA may be transferred from one bacterial cell to another in the form of a plasmid. Plasmids may carry genetic information in addition to the information contained on a chromosome, which bacteria may use under special conditions. For instance, plasmids may carry the genetic information for antibiotic resistance, virulence, bacteriocins, and metabolic activity (Madigan et al., 2000). Transduction is the process in which a part of a donor chromosome is packaged into a phage head and transferred by viruses. If the virus packaging mechanism selects genes that confer antibiotic resistance, then resistance may be spread to bacterial cells infected by the viruses. Transformation is the process in which cells take up free DNA from the environment (Snyder and Champness, 1997). If the DNA contains antibiotic resistance genes, then antibiotic resistance may be conferred to the transformant. Thus the transformant now has the genetic material encoding antibiotic resistance.

Amoxicillin

Amoxicillin is an orally absorbed broad-spectrum antibiotic with a variety of clinical uses including ear, nose, and throat infections and lower respiratory tract infections. As a chemical modification of ampicillin, which is poorly absorbed after oral administration, amoxicillin is better absorbed by the gastrointestinal tract than ampicillin (Sum et al., 1989). Amoxicillin is prescribed for the treatment of infections of beta-lactamase-negative stains, which are bacterial strains that do not possess the ability to produce beta-lactamase enzymes. Figure 2 presents the chemical structures of amoxicillin (R=0H) and penicilloic acid, a transformation product produced during beta-lactam ring cleavage.

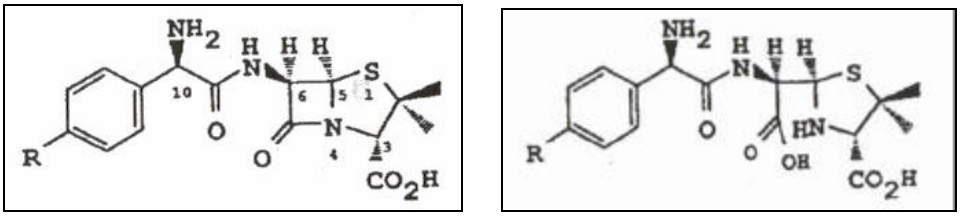

Figure 2. Chemical structure of amoxicillin (left) and penicilloic acid (right) (Connor et al., 1994).

Amoxicillin is a semi-synthetic penicillin obtaining its antimicrobial properties from the presence of a beta-lactam ring. Amoxicillin and other penicillin-like antibiotics target bacterial cell walls. Beta-lactam antibiotics bind to and inhibit the enzymes needed for the synthesis of peptidoglycan, a component of bacterial cell walls. As bacteria multiply and divide, the defective walls cannot protect the organism from bursting in hypotonic environments and cell death occurs.

Many mechanisms exist for resistance to beta-lactam antibiotics. Resistance is considered an increase in the minimal inhibitory concentration (MIC) of the antibiotic, which could be the result of many different mechanisms, whereas tolerance does not alter the bacteria's susceptibility to the drug but improves bacterial survival during treatment. For optimal bactericidal action, the dose must be greater than the organism's MIC. In the case of beta-lactam antibiotics, this dose is approximately four to five times the MIC. When antibiotic concentration is less then the MIC, bacteria recover from the exposure and begin growth (Ronchera, 2001). When the drug is prescribed to a patient with the infection, the dose will be greater than the MIC. However, it is unlikely that wastewater containing urine and feces will have antibiotic concentrations greater than the MIC; therefore, antimicrobial effects will probably not be observed. However, low concentrations of antibiotics encourage the development of antibiotic resistance. Thus, wastewater streams containing urine and feces likely aid in the development of antibiotic resistance. In *S. aureus*, which is a major human pathogen, three mechanisms of beta-

lactam resistance have been identified: (1) beta-lactamase-mediation inactivated through hydrolysis of the beta-lactam nucleus, (2) penicillin-binding proteins (PBP)-associate intrinsic resistance due to the lower of the affinity of PBPs or the acquisition of new PBPs, and (3) tolerance of the beta-lactam antibiotic as a result of autolysins inhibition (Georgepapadakou et al.,1988). PBPs are the enzymatic targets of beta-lactam antibiotics. Beta-lactam resistance due to the alteration of PBPs has been detected in many isolates as well as most of the major human invasive pathogens (Tomasz, 1988).

For Gram-negative organisms, such as *E. coli* and nitrifying organisms, another mechanism of resistance to beta-lactam antibiotics, including amoxicillin, is the hindering of diffusion of the antibiotic by the outer membrane, which acts as a permeability barrier (Frere and Joris, 1988). Antibiotics must pass through porins, which are non specific outer membrane channels. The antibiotics ability to pass through porins depends on the size, hydrophobicity, and charge of the antibiotic (Danziger and Pendland, 1995). In addition, the outer membrane prevents the leaking of beta-lactamases into the culture environment (Frere and Joris, 1988). All bacteria may be divided into Gram-positive and Gram-negative organisms. The classification was developed by Gram, which is based on a dye procedure; the color of the dyed bacteria is related to the composition of bacterial cell walls. Gram-positive organisms appear blue following a Gram stain, and they posses a thick layer of peptidoglycan and no outer membrane. Beta-lactam antibiotics easily penetrate the thick layer of peptidoglycan in Gram-positive bacteria (Danziger and Pendland, 1995). Gram-negative organisms have an outer membrane and a thin layer of peptidoglycan inside the periplasmic space and are stained red in a Gram stain. Figure 3 is a drawing of the cell wall structures of Gram-negative and Gram-positive bacteria.

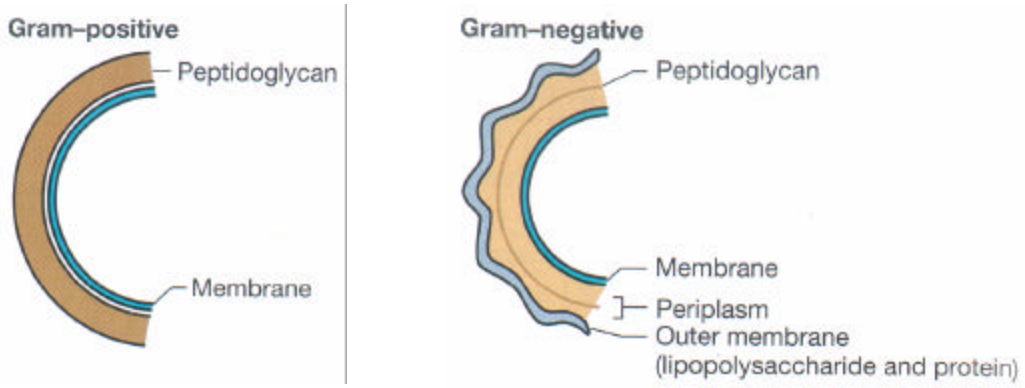

Figure 3. Structure of Gram-positive and Gram-negative bacteria (Madigan et al., 2000).

As mentioned previously, beta-lactamases are enzymes that cleave the beta-lactam ring and render the antibiotic useless. The genetic information for beta-lactamases is contained on either plasmids or chromosomes; however, genes for resistance are usually carried by plasmids. Beta-lactamase production may be either constitutive or inducible. Constitutive production results in a constant level of beta-lactamase production, which is independent of exposure to antibiotics. If beta-lactamase production is inducible, then beta-lactamases are produced following exposure to a signal, such as a beta-lactam antibiotic. Furthermore, production of the beta-lactamases ceases when the bacterium is no longer exposed to the signal (Danziger and Pendland, 1995). Beta-lactamases are classified according to (1) their genetic location (chromosome vs. plasmid), (2) gene expression (inducible vs. constitutive), (3) microorganism, (4) inhibition by beta-lactamase inhibitors, and (5) substrate. Figure 4 presents beta-lactamases and their distribution in nature.

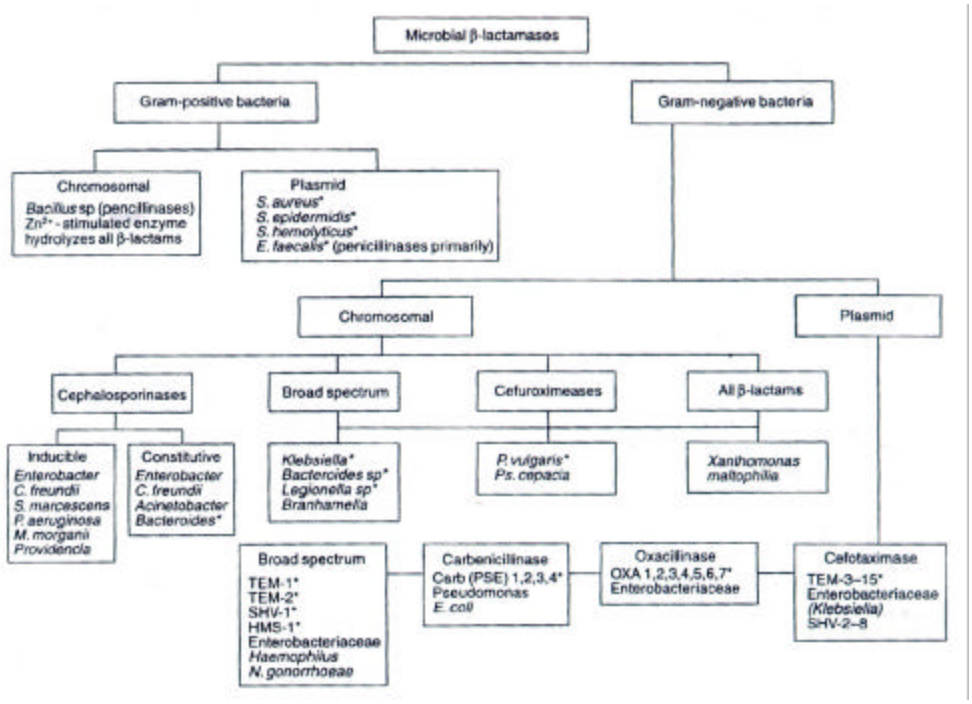

Figure 4. Beta-lactamases and their distribution in nature (Danziger and Pendland, 1995).

To reduce the potential for beta-lactam cleavage, beta-lactamase inhibitors are frequently combined with beta-lactam antibiotics. The purpose of the beta-lactamase inhibitors is to prevent the beta-lactamases from inactivating the antibiotic thereby increasing the effectiveness of the antibiotic. Examples of beta-lactamase inhibitors are sulbactam, clavulanate, and tazobactam (Danziger and Pendland, 1995). In many cases, amoxicillin is combined with clavulanic acid, a beta-lactamase inhibitor.

Antibiotics in the Environment

Drug residues, including antibiotics, have been observed in various aquatic environments including groundwater, surface water, and tap water (Alvero, 1987; Campeau et al., 1996). Sources of antibiotics include the treatment of human infections, veterinary use (e.g., animal feeding operations), aquaculture, and land application of compost containing sludge from wastewater treatment plants. In human uses, which will be the primary focus of this paper, antibiotics enter waste streams through feces and urine. To demonstrate, Hoeverstadt et al. (1986) detected several antibiotics in human feces, including trimethoprim and doxycycline in concentrations ranging from 3 to 40 mg/kg and erythromycin concentrations from 200 to 300 mg/kg. The concentration of antibiotics in urine is dependent on dosage, type of dosing (intravenous, intramuscular, or oral), food and beverage consumption, and elapsed time since dosage (Mastrandrea et al., 1984). In addition, absorption is also a property of the antibiotics. For example, amoxicillin is a chemically modified form of ampicillin and the modifications improve its absorption characteristics.

Extreme difficulties arise in estimating the mass of antibiotics entering the environment. In general, records containing the quantity of antibiotics prescribed annually are incomplete and the data available varies from country to country. Furthermore, it is unknown if the medication is taken as prescribed. Absorption rates vary for each individual further complicating the estimate of antibiotics entering the environment. Therefore, researchers have begun analyzing environmental samples for the presence of antibiotics. Table 1 presents the concentration of antibiotics present in secondary effluent and surface water in Germany.

Table 1. Concentrations of selected antibiotics applied in Germany (Zwiener et al., 2001).

Antibiotic	Prescribed Mass (tons/yr)	Secondary Effluent Concentration (µg/L)	Surface Water Concentration (µg/L)
Clarithromycin	1.3-2.6	0.24	0.26
Erythromycin	3.9-19.8	6.00	1.70
Roxithromycin	3.1-6.2	1.00	0.56
Chloramphenicol	--	0.56	0.06
Sulfamethoxazole	16.6-76	2.00	0.48
Trimethoprim	3.3-15	0.66	0.20

In the United States, the U.S. Geological Survey completed a study that measured the concentrations of 95 organic wastewater contaminants (OWCs) in water samples from 139 streams in thirty states during 1999 and 2000 (Kolpin et al., 2002). OWCs include pharmaceuticals, hormones, and other organic contaminants. The compounds detected represented a wide range of residential, industrial, and agricultural sources. The most frequently detected compounds were coprostanol (fecal steroid), cholesterol (animal and plant steroid), insect repellant (N,N-diethyltoluamide), caffeine, triclosan (antimicrobial disinfectant), fire retardant (tri(2-chloroethyl)phosphate), and a nonionic detergent metabolite (4-nonylphenol). In addition to these compounds, 31 veterinary and human antibiotic and antibiotic metabolites were investigated. Fourteen of the 31 antibiotics were not detected in this study. Table 2 contains the antibiotic, frequency of detection, maximum detected concentration (µg/L), and median detected (µg/L) concentration of the remaining 17 antibiotics.

Table 2. Summary of antibiotics in streams of the U.S. (Kolpin et al., 2002).

Antibiotic	Number of Samples	Reporting Level (µg/L)	Frequency (%)	Max (µg/L)	Median (µg/L)
Chlortetracycline (1)	84	0.10	2.4	0.69	0.42
Ciprofloxacin	115	0.02	2.6	0.03	0.02
Erythromycin-H_20	104	0.05	21.5	1.7	1.0
Lincomycin	104	0.05	19.2	0.73	0.06
Norfloxacin	115	0.02	0.9	0.12	0.12
Oxytetracycline (2)	84	0.10	1.2	0.34	0.34
Roxithromycin	104	0.03	4.8	0.18	0.05
Sulfadimethozine (2)	84	0.05	1.2	0.06	0.06
Sulfamethazine (1)	104	0.05	4.8	0.12	0.02
Sulfamethazine (2)	84	0.05	1.2	0.22	0.22
Sulfamethizole (1)	104	0.05	1.0	0.13	0.13
Sulfamethoxazole (1)	104	0.05	12.5	1.9	0.15
Sulfamethoxazole (3)	84	0.023	19	0.52	0.066
Tetracycline (2)	84	0.10	1.2	0.11	0.11
Trimethoprim (1)	104	0.03	12.5	0.71	0.15
Trimethoprim (3)	84	0.014	27.4	0.30	0.013
Tylosin (1)	104	0.05	13.5	0.28	0.04

Several studies have identified antibiotics in wastewater treatment plant (WWTP) flow streams and in WWTP effluents (Stelzer et al., 1985; Grabow et al., 1976; Bell, 1979; Misra et al., 1979; Radtke and Gist, 1989; Malik and Ahmad, 1994) at concentrations from ng/L to µg/L. Alder et al. (2000) detected up to 0.8 µg/L of cirpofloxacin in a WWTP effluent and 0.01 to 0.29 µg/L in the WWTP influent. Hirsch

et al. (1999) found erythromycin concentrations up to 6 µg/L in WWTP effluent. Ciprofloxacin was observed in hospital effluent at concentrations between 3 and 89 µg/L, which is significantly higher than concentrations presented in other studies. Amoxicillin concentrations in wastewater from a German hospital were between 28 and 82.7 µg/L (Henninger et al., 2000). Peniciloly groups were observed at concentrations greater than 25 ng/L and 10 µg/L in river water and potable water, respectively (Halling-Sorensen et al., 1998). Therefore, wastewater treatment plants (WWTPs) are receiving wastes that contain low concentrations of antibiotics. Exposure to small concentrations of antibiotics selects for organisms resistant to antibiotics. Subsequently, WWTPs may be a reservoir of antibiotics as well as antibiotic resistant bacteria.

Antibiotic Resistance

Antibiotic resistance has been observed in various aquatic environments including river and costal areas, domestic sewage, surface water and sediments, lakes, sewage polluted ocean water, and drinking water (Merzioui and Baleux, 1994). These aquatic environments represent a variety of ecosystems and may include a variety of climates. The consequences of antibiotic resistant organisms may be different for each environment.

WWTPs are used to treat domestic and industrial wastewater so that it may be disposed in the natural aquatic environment, including rivers, lakes and streams, with minimal impact on aquatic life. Currently, the WWTP effluent must meet regulatory limits for suspended solids, nutrients, fecal coliforms, total coliforms, and a biological oxygen demand; however, regulatory limits have not been developed for antibiotic agents and the effect of low antibiotic concentrations and antibiotic resistance development receives limited attention. The role of WWTPs on the spread of antibiotic resistance to the natural environment is an important key to the ecological impact of human discharges.

Antibiotic Resistance in WWTP Influent

WWTPs typically accept discharges from hospitals and may receive discharges from pharmaceutical plants. Guardabassi et al. (1998) investigated the antibiotic

resistance of *Acintobacter* spp. in sewers receiving waste from a hospital and pharmaceutical plant. The level of susceptibility to six antimicrobial agents was determined in 385 *Acinetobacter* strains isolated from samples collected up stream and downstream from the hospital and pharmaceutical plant. The antimicrobial agents analyzed include amoxicillin, oxytetracycline, chloramphenicol, sulfamethoxazole, gentamicin, and ciprofloxacin. A prevalence of oxytetracycline resistance was observed to increase in the sewer as the result of hospital discharge; however, the level of resistance decreased downstream of the discharge.

Antibiotic Resistance in WWTPs and Their Discharges

The incidence of outbreaks involving waterborne antibiotic-resistant bacteria has led to a serious problem of the death of patients who do not respond to antibiotics. One source of antibiotic-resistant bacteria in the environment is effluent from WWTPs (Hassani et al., 1992). The purpose of the Hassani et al. (1992) study was to evaluate the distribution of *Aeromonas* species present in wastewater treatment ponds to determine the effect of treatment on drug resistance incurred by the species. The importance of evaluating *Aeromonas* species is that they are a broad group of organisms commonly found in aquatic environments. During the course of this 17-month study, the distribution of the *Aeromonas* observed in the system differed between the cold and warm months. The most common species of *Aeromonas* observed were *A. caviae, A. hydrophila,* and *A. sobria*. Seven antibiotics (amoxicillin, cephalothin, streptomycin, trimethoprim-sulfamethoxazole, chloramphenicol, polymyxin B, and nalidixic acid) were tested on 264 isolates in this study. All of the isolates were resistant to amoxicillin and 73 percent exhibited resistance to cephalothin, both of which are beta-lactam antibiotics. The overall frequency of multiple antibiotic resistances among bacterial isolates was 77 percent and the antibiotic resistance index for the total strains was 0.29. Temperature appeared to have an effect on multiple-drug resistance. During the warm months, the level of resistance was greater in the bacteria isolated from the influent than those isolated in the effluent from the pond. Overall, the *A. sobria* were more susceptible to the antibiotics investigated in this study than either *A. caviae* or *A. hydrophila*. In addition, each species exhibited different resistance patterns than the other species. For example,

the resistance to cephalothin of *A. caviae, A. hydrophila* and *A. sobria* were 91, 96 and 9 percent, respectively.

Another study evaluated the effect of wastewater stabilization ponds on antibiotic resistance on *Aeromonas* (Imzilin et al., 1996). Differences in resistance patterns of *Aeromonas* isolated from the raw sewage and stabilization pond effluent were not observed. All strains possessed multiple resistances, including resistance to ampicillin, amoxicillin, and novobiocin. Approximately 90 percent of the strains of *A. hydrophila* and *A. caviae* were resistant to cephalothin, and almost 80 percent of the *A. sobria* were susceptible. The results of this study are fairly similar to the results obtained from the study by Hassani et al. (1992).

Mezriou and Baleux (1994) investigated the antibiotic resistance of 879 *E. coli* strains isolated from raw domestic sewage and the effluent from aerobic lagoons and activated sludge plants. Both aerobic lagoons and activated sludge plants are used to reduce the BOD_5 leaving the treatment facility. The results of this study indicate that the aerobic lagoons were effective in removing fecal coliforms in the wastewater, but the system selected for antibiotic resistant *E. coli* by selecting for *E. coli.* The number of antibiotic resistant strains of *E. coli* in the effluent increased as compared to the influent. For both the inflow and outflow, the incidence of antibiotic resistance increased as the number of antibiotics was reduced from seven to one. The maximum polyresistance for a strain was seven antibiotics (ampicillin, mezlocillin, gentamicin, netilmicin, tobramycin, doxycyclin, and chloramphenicol). The level of antibiotic resistant *E. coli* strains in the outflow of the activated sludge was not constant and did not appear to develop in a manner similar to the aerated lagoon. In both the activated sludge and aerated lagoon system, resistance to quinolones and aminosides was not observed.

WWTP Discharges and Their Effect on the Natural Environment

To evaluate the impact of urban effluent, including WWTP discharges, Goni-Urriza et al. (2000) investigated antibiotic resistance in bacteria isolated from the Arga River in Spain. River samples were collected upstream and downstream of the water discharged from the city of Pamplona's WWTP. *Enterobacteriaceae*, from human and animal commensal flora, and *Aeromonas* were investigated. Most *Aeromonas* (72

percent) and 20 percent of the *Enterobacteriaceae* were resistant to nalidixic acid, which is a quinolone. The rate at which antibiotic resistances decreased downstream from the discharge was similar for the two groups of bacteria. Genetic analysis indicated that these resistances were mostly chromosomal mediated for *Enterobacteriaceae* and exclusively chromosomally mediated for *Aeromonas*. Other studies have observed less resistance of native and fecal bacteria upstream of urban areas and WWTP discharges, increased resistance immediately downstream of urban areas and WWTP discharges, and decreased resistance farther downstream (Boon and Cattanach, 1999; Pathak et al., 1993; Iwane et al., 2001). Another study by Gonzalo et al. (1989) evaluated antibiotic resistance and virulence factors of 418 *E. coli* strains isolated from river water receiving sewage discharge. The data indicated that bacteria from less contaminated water present less antibiotic resistance and virulence factors than those isolated from highly contaminated water. The results suggest that antibiotic resistance and virulence factors do not survive well in environments without selective pressure.

Natural aquatic environments, including lakes, rivers, and streams are environments in which antibiotic resistance may be developed. Arvanitdou et al. (1997) investigated the transfer of antibiotic resistance among *Salmonella* strains isolated from surface waters in northern Greece. Differences in antibiotic use and climate conditions resulted in geographic variations of antibiotic resistance among bacteria in surface water. The study showed that 24 percent of the *Salmonella* strains tested showed resistance to one or more of the antibiotics tested. Resistance to streptomycin was most common but was not transferable in all cases. However, ampicillin resistance (ampicillin is a beta-lactam antibiotic) was transferable. The authors believed these findings supported the presence of a common plasmid-mediated TEM type beta-lactamase. In one case, ampicillin resistance was cotransferred with resistance to aminoglycosides. Bacteria of non-fecal origin in natural aquatic environments free of natural anthropogenic influence demonstrated antibiotic resistance to one or more antibiotics; resistance may not be plasmid-mediated (Magee and Quinn, 1991).

Antibiotic Resistance Transfer

One of the greatest concerns of antibiotic resistance is the spread of antibiotic resistance from one bacterial species to another, especially in the case of resistance transfer between nonpathogenic to pathogenic bacteria. Antibiotic resistance has been a concern in an institutionalized environment such as a hospital; however, it may also be a concern in aquatic environments such as wastewater treatment systems. Resistance to newer beta-lactam agents was observed between *Klebsiella pneumoniae* and *E. coli* in a hospital in France, as observed by a decreased susceptibility of *E. coli* to cefotaxime. Three beta-lactamases were identified mediating cefotaxime resistance as well as penicllin and other cephalosporin resistance. Therefore, these beta-lactamases were termed extended broad-spectrum beta-lactamases (Jarlier et al., 1988).

Evidence suggests that healthy members of a community may contain a reservoir of bacterial antibiotic resistance genes even in commensal flora (Shanahan et al., 1994). These resistance reservoirs may complicate treatment of infections by invading pathogens who transfer resistance to nonpathogens. In Gram-negative bacteria, resistance is commonly mediated by TEM-1 beta-lactamases, which have been shown to account for up to 80 percent of all plasmid-mediated resistance. In Edinburgh, U.K., antibiotic resistance was observed in healthy human subjects, including resistance to ampicillin. Plasmids containing TEM-1 beta-lactamases encoding information were present throughout the community and were believed to be culprit of many extended-spectrum beta-lactamases.

Summary

These studies indicate that industrial and domestic discharges may affect the antibiotic resistance patterns observed in a WWTP. Furthermore, WWTPs have the ability to alter the antibiotic resistance patterns of bacteria in ecosystems containing the WWTP outfall. As a consequence, environmental bacteria, pathogens, and non-pathogens may confer resistance to currently prescribed antibiotics.

Lubbock Water Reclamation Plant

The Lubbock Water Reclamation Plant (LWRP), located in Lubbock, Texas, served as the test facility for the fate of amoxicillin in a full-scale wastewater treatment

plant. The flow rate for the LWRP is approximately 20 MGD. Figure 5 is a flow diagram of the LWRP. Note that there are three process streams for the plant, which have been the result of plant expansions over the many years of operation. Primary treatment of the influent to the plant consists of screening and grit removal. After primary treatment, the flow streams are split before secondary treatment. For secondary biological treatment, the plant employs activated sludge in Plants 3 and 4. Plant 2 uses biotowers for secondary treatment. The facility does not employ tertiary removal. Instead, the effluent is used to irrigate farmland, discharged to Yellow House Canyon, or sent to XCEL. Sludge from secondary treatment is thickened, digested in anaerobic digesters, dewatered and landfilled.

The fate of amoxicillin in the LWRP was determined by measuring the ambient concentrations of amoxicillin at four locations in the plant over nine months. The objective of this experiment is to investigate the fate of amoxicillin in a full-scale wastewater treatment plant. Due to the dilution of urine by other wastewater streams entering a full-scale wastewater treatment plant as well as biotic activity in sewer systems, amoxicillin concentrations were expected to be near the detection limit in the influent and effluent of the plant, respectively.

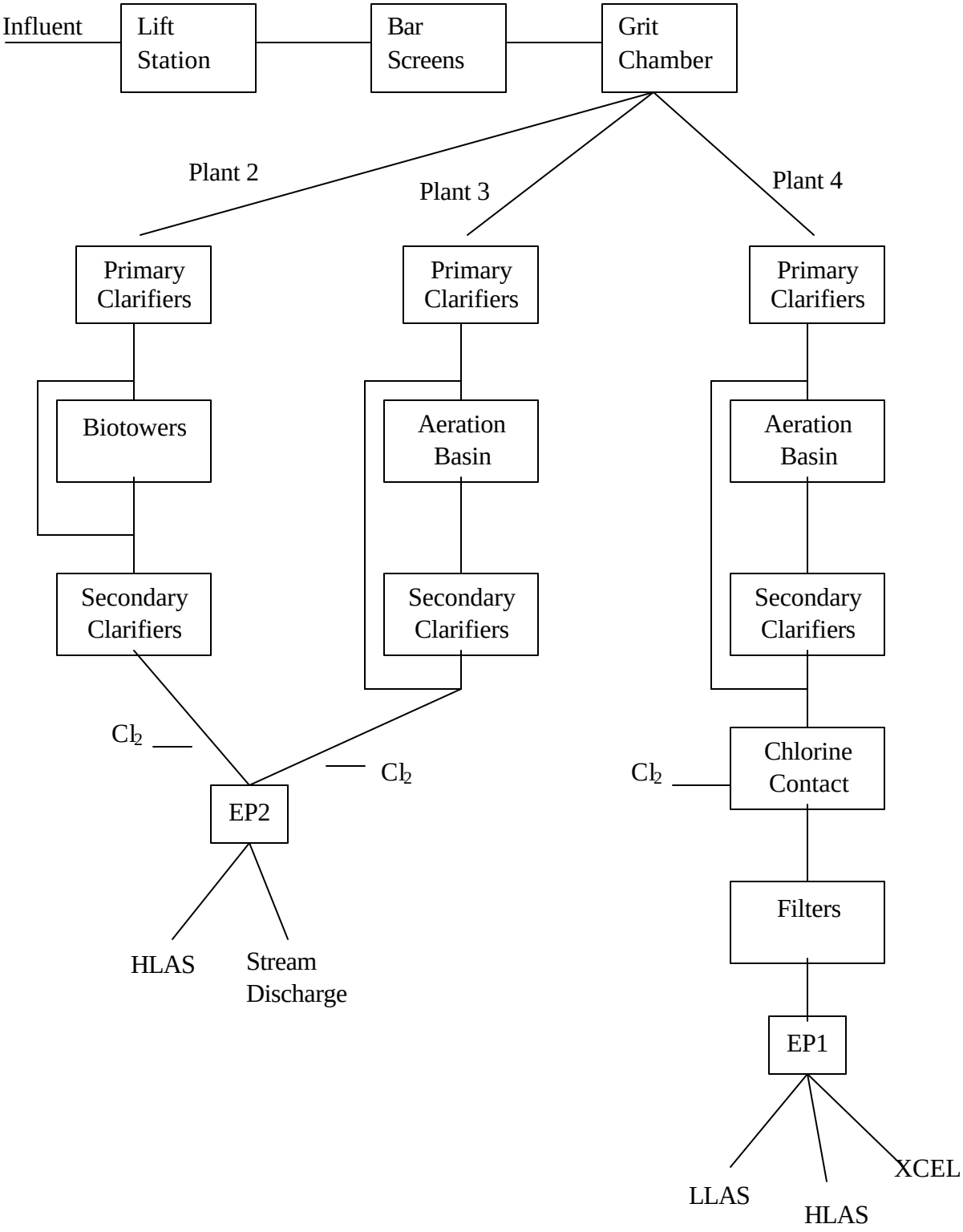

Figure 5. Schematic of the LWRP.

EXPERIMENTAL PROCEDURE

Fate of Amoxicillin in a Water Reclamation Plant--Lubbock, TX

Wastewater samples were collected from the influent, primary sludge, activated sludge basin, and the effluent of the Plant 4 of the LWRP. Samples were collected by LWRP personnel on the second Friday of each month from May to December. Samples were taken immediately to the ESL and filtered using a 0.45 μm filter. The primary sludge samples were centrifuged before filtering. The wastewater samples were filtered to prevent clogging of the C18 cartridges, which were required by the amoxicillin preparation procedure. All amoxicillin wastewater samples were analyzed in triplicate.

Antibiotic Resistance

The change in antibiotic resistance in the LWRP was investigated at four locations in the plant over eight months. To investigate the occurrence of antibiotic resistance in microorganisms in the systems examined in this research the disk diffusion susceptibility test was performed on samples obtained from the systems.

To investigate antibiotic resistance of the bacteria used in LWRP, samples were collected from the influent, activated sludge tank, primary sludge and effluent. Samples were collected, by LWRP personnel on the second Friday of each month from May to December in sterile, 1-L bottles. The samples were immediately taken to the ESL and analyzed.

The disk diffusion susceptibility test was performed to determine if heterotrophic organisms in the LWRP samples were resistant to multiple antibiotics. The LWRP samples were plated on nutrient agar and Hinton-Mueller plates. The antibiotics investigated in this study include amoxicillin with clavulanic acid and three other beta lactam antibiotics: penicillin, ampicillin, and cephalothin. Other antibiotics investigated in this study were bacitracin, ciprofloxacin, rifampin, streptomycin, tetracycline, and vancomycin. Table 3 lists the antibiotics and their concentrations used in this study. Table 4 presents a summary of the antibiotics investigated and their mechanisms.

Table 3. Antibiotics and concentrations of susceptibility disks.

Antibiotic	Concentration (µg)
Amoxicillin with clavulanic acid	30
Ampicillin	10
Bacitracin	10
Penicillin (units)	10
Cephalothin	30
Ciprofloxacin	30
Rifampin	5
Streptomycin	10
Tetracycline	30
Vancomycin	30

To create a lawn of bacteria, 0.1 mL of the wastewater was spread on the agar. After an incubation period, the zone of inhibition, which is the area around the disk without bacterial growth, was measured. The size of the zone of inhibition determined the susceptibility of the organisms to the antibiotic. The diameter of the paper disks were 0.7 cm. If the diameter of the zone of inhibition was between 0.7 and 1.0 cm, the organisms were considered resistant, because the organisms grew up to the disk. If the zone was between 1.0 and 1.2 cm, the bacteria were considered moderately susceptible and if the zone was greater than 1.2 cm, the microorganisms were considered susceptible. All samples were conducted in triplicate. Plates were incubated for 24 hours at 30^{o}C.

Media Preparation and Sterilization Procedures

Nutrient agar and Hinton-Mueller agar were used in this experiment. All of these products were prepared according to the manufacturer's directions.

Table 4. Summary of antibiotics and resistance mechanisms (Kimball, 2001).

Antibiotic	Class	Mechanism
Amoxicillin	Beta-lactam	Cell wall synthesis (PBPs)
Ampicillin	Beta-lactam	Cell wall synthesis (PBPs)
Bacitracin		Cell wall synthesis
Cephalothin	Beta-lactam	Cell wall synthesis (PBPs)
Ciprofloxacin	Quinolones	DNA gyrase inhibitor
Penicillin	Beta-lactam	Cell wall synthesis (PBPs)
Rifampin	Rifampin	Bacterial RNA Polymerase
Streptomycin	Aminoglycosides	70s ribosome subunit
Tetracycline	Tetracycline	30s ribosome subunit
Vancomycin	Glycopeptide	Cell wall synthesis (D-alanines)

Sterilization

Liquids, laboratory supplies, and media, when specified, were sterilized using an autoclave. The autoclave was operated for 15 minutes at 212^{o}F. The pressure was adjusted for Lubbock's elevation. Glassware was sterilized by dry sterilization, which requires glassware to be baked in an oven at 180^{o}C for four hours. Amoxicillin solution was sterilized by filtering the solution with a 0.22 µm filter.

Amoxicillin Quantification

A method developed by Sorenson and Snor (2001) was used to quantify the concentration of amoxicillin in the wastewater samples analyzed. Two milliliters of the sample was added to 20 mL of phosphate buffer 9.0 and mixed. Using a vacuum manifold, SPE cartridges (Waters) were washed with 2.0 mL of methanol and 5 mL of water. The samples were drawn through the cartridges at a flow rate of 2.0 mL/min. The column was washed three times with 2 mL of phosphate buffer 9.0. The samples were vacuumed dried for 1 minute. The cartridges were eluted with 2.5 mL of acetonitrile, which was collected in 15 mL polypropylene tubes and evaporated to dryness under nitrogen at a temperature of 55^{o}C. The residue was redissolved in 600 µL of phosphate buffer 9.0 and centrifuged filter through a cellulose membrane filter at 3000 x g for 15

minutes. Then, 500 µL of the filtrate was transferred to a 15 mL polypropylene tube. Next, 75 µL of derivitization reagent I was added and the sample was vortex-mixed for 30 seconds. After 10 minutes, 450 µL of derivitization reagent II was added and the sample was vortex-mixed for 60 seconds. The samples were placed in a water bath (55^{o}C) to react for 30 minutes. The samples were cooled in cool water and transferred to vials. The samples were quantified using an HPLC. The injection volume was 500 µL and the mobile phase flow-rate was 1.0 mL/min. The detection wavelength was 323 nm. The analytical instrument was calibrated on each day of analysis, and QC samples were run at a maximum of 20 samples, followed by blanks, to ensure that the instrument was still calibrated correctly.

RESULTS AND DISCUSSION

Fate of Amoxicillin in a Water Reclamation Plant--Lubbock, TX

To investigate the concentration of amoxicillin in a full-scale wastewater treatment plant, wastewater samples were collected from the Lubbock Wastewater Reclamation Plant (LWRP) in Lubbock, Texas. Samples were collected on the second Friday of every month between May and December 2002. The samples were immediately taken to the ESL and analyzed for amoxicillin and antibiotic resistance. All samples were analyzed in triplicate. The purpose of this experiment was to monitor the fate of amoxicillin in the LWRP to determine if amoxicillin was present in the plant's influent and effluent.

During the eight-month experiment, amoxicillin was detected in the influent of the plant only in the May samples; amoxicillin was not detected at any other sample locations during any of the months of this study. Table 5 presents a summary of the amoxicillin analysis. NA indicates not applicable. Due to equipment problems, samples could not be analyzed in December.

Table 5. Amoxicillin concentrations in the LWRP.

	Amoxicillin Concentration (mg/L)			
Month	Influent	Primary Sludge	Activated Sludge	Effluent
May	0.15	<0.10	<0.10	<0.10
June	<0.10	<0.10	<0.10	<0.10
July	<0.10	<0.10	<0.10	<0.10
August	<0.10	<0.10	<0.10	<0.10
September	<0.10	<0.10	<0.10	<0.10
October	<0.10	<0.10	<0.10	<0.10
November	<0.10	<0.10	<0.10	<0.10
December	NA	NA	NA	NA

During the entire course of the experiment, amoxicillin was not detected in the effluent of the LWRP. The results suggested that amoxicillin may not represent an

environmental concern. This supported the hypothesis that amoxicillin would not be present at levels that may exert an impact on reclaimed wastewater end-users or aquatic life.

Antibiotic Resistance in the LWRP

A summary of the results of the antibiotic resistance tests performed using the LWRP wastewater are presented in Tables 6 through 9. Due to the large volume of data collected, a summary of the data is presented herein and the remaining data are presented in the appendices of this document. In the tables, S indicates the bacteria were susceptible to the antibiotic, MR indicates the bacteria were moderately resistant, and R indicates the bacteria were resistant to the antibiotic. In general, the bacteria in the plant were resistant to the beta-lactam antibiotics, including penicillin, ampicillin, and cephalothin. Bacteria in the influent were more resistant to the antibiotics examined than in the other flow streams. Bacteria in the plant were usually resistant to bacitracin and vancomycin. The resistance to amoxicillin with clavulanic acid, streptomycin, rifampin, and ciprofloxacin varied monthly.

The data showed that antibiotic resistance patterns changed in the plant, which may be a consequence of the organisms present and the fluctuations of organism population (Hassani et al., 1992; Imzilin et al., 1996). Of particular interest to this study was the resistance to the beta-lactam antibiotics. The organisms were resistant to the beta-lactam antibiotics. Generally, bacteria grew completely up to the disk. The resistance observed in the LWRP complemented the results obtained from the JSC-WRS and the TTU-WRS. Both systems illustrated resistance to the beta-lactam antibiotics. The addition of the beta-lactamase inhibitor, clavulanic acid, slightly reduced (moderately resistant versus resistant) the organisms' resistance to the amoxicillin, as compared to the resistance patterns of other beta-lactam antibiotics.

In addition, the bacteria exhibited different antibiotic resistant mechanisms. Table 4 contained the antibiotic and the mechanism of disinfection. The results of this experiment indicated the prevalence of antibiotic resistance in the LWRP and possible health effects and concerns to the ecosystems and end users of the water containing LWRP discharges. The concern of antibiotic resistance transfer from bacteria in the

effluent of the LWRP to bacteria in the surrounding environment is a concern, especially if antibiotic resistance is spread to pathogenic bacteria.

Table 6. Antibiotic Resistance in the Influent of the LWRP.

Antibiotic	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Amoxicillin with clavulanic acid	R	R	R	MR	S	S	R
Ampicillin	R	R	R	R	R	R	R
Bacitracin	R	R	R	NA	R	R	R
Cephalothin	R	R	R	R	R	R	R
Ciprofloxacin	S	R	R	S	R	S	MR
Penicillin	R	R	R	R	R	R	R
Rifampin	R	R	MR	MR	R	R	R
Streptomycin	MR	R	MR	R	S	R	R
Tetracycline	NA	R	R	R	NA	NA	NA
Vancomycin	R	R	R	R	R	R	R

Table 7. Antibiotic Resistance in the Primary Sludge of the LWRP.

Antibiotic	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Amoxicillin with clavulanic acid	MR	S	MR	MR	MR	R	R
Ampicillin	R	R	R	R	R	R	R
Bacitracin	R	R	R	NA	R	R	R
Cephalothin	R	R	R	R	R	R	R
Ciprofloxacin	MR	S	MR	S	MR	MR	S
Penicillin	R	R	R	R	R	R	R
Rifampin	R	MR	MR	R	R	R	MR
Streptomycin	MR	MR	S	R	R	R	R
Tetracycline	NA	R	MR	R	NA	NA	NA
Vancomycin	R	R	R	R	R	R	R

Table 8. Antibiotic Resistance in the Activated Sludge of the LWRP.

Antibiotic	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Amoxicillin with clavulanic acid	S	R	S	MR	S	S	S
Ampicillin	R	R	S	R	R	R	R
Bacitracin	R	R	R	NA	R	R	R
Cephalothin	R	R	R	R	R	R	R
Ciprofloxacin	S	R	S	S	R	S	MR
Penicillin	R	R	R	R	R	R	R
Rifampin	S	MR	MR	S	R	R	R
Streptomycin	MR	R	S	R	S	MR	S
Tetracycline	NA	R	S	MR	NA	NA	NA
Vancomycin	R	R	R	R	R	R	R

Table 9. Antibiotic Resistance in the Effluent of the LWRP.

Antibiotic	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Amoxicillin with clavulanic acid	S	S	S	R	S	S	S
Ampicillin	R	R	MR	R	S	S	S
Bacitracin	R	R	R	NA	R	R	R
Cephalothin	R	R	MR	R	MR	MR	R
Ciprofloxacin	S	R	S	R	S	S	S
Penicillin	R	R	R	R	MR	R	R
Rifampin	MR	MR	S	R	R	S	MR
Streptomycin	S	S	R	R	S	S	S
Tetracycline	NA	S	S	R	NA	NA	NA
Vancomycin	R	R	S	R	R	R	R

In the LWRP, bacteria were resistant to multiple antibiotics. The greatest concern was that antibiotic resistant bacteria in the effluent of the LWRP may spread the genetic information encoding antibiotic resistance to organisms in the environment of the LWRP outfall. As a consequence, changing the antibiotic resistance properties of the bacteria in an ecosystem may disrupt the ecosystem, and the water may be a health hazard to end-users. For example, say a person uses the LWRP water to irrigate his/her farmland. During a visit at the farm, the farmer cuts their hand and the reclaimed wastewater comes into contact with the wound. Bacteria present in the water may infect the cut and the farmer may be hospitalized with a difficult infection to cure. This scenario is possible with the use of reclaimed wastewater. One solution to the aforementioned situation is to thoroughly disinfect the wastewater before releasing to the environment or the end users. This will minimize the bacterial population in the wastewater and minimize the exposure of the receiving ecosystem to antibiotic resistant bacteria.

Amoxicillin was detected in the influent of the LWRP on only one occasion during the eight-month study. Amoxicillin was not detected in the plant's flow streams (primary sludge, activated sludge or in the LWRP effluent). Due to the dilution of toilet flush water, 28 percent of interior residential water use (Metcalf and Eddy, 1991) with other domestic and municipal water flow streams, the concentration of amoxicillin is anticipated to be at or below the detection limit. In addition, it is unlikely that everyone served by the LWRP would be on antibiotics. Higher concentrations of amoxicillin may be observed in the effluent of a hospital or other medical facility; however, samples of this nature were not collected in this research. Based on the results of this study, amoxicillin, when present, is be believed to be degraded in the microbially-active sewer systems that transmit wastewater from the producers to the wastewater treatment plant. Thus, it is unlikely that amoxicillin would be present in the influent of the LWRP at concentrations greater than detected in this study.

CONCLUSIONS AND RECOMMENDATIONS

A recent concern of reusing wastewater for consumption is the presence of chemical contaminants, including a new category of compounds: personal care products and pharmaceuticals. Antibiotics are an especially troubling class of compounds due to their ability to produce antibiotic resistance in bacterial populations. Antibiotics enter the environment from a variety of sources including discharges from domestic wastewater treatment plants and pharmaceutical companies, runoff from animal feeding operations, infiltration from aquaculture activities, leachate from landfills, and leachate from compost made of animal manure containing antibiotics. However, antibiotics are not confined to the natural aquatic environment. Detectable concentrations of antibiotics have been observed in tap water (Herberer et al., 1998; Masters, 2001). The startling fact is that these compounds are passing through water treatment processes and contaminating drinking water supplies. The concentrations of these contaminants typically range from nanogram/liter (ng/L) to microgram/liter (µg/L); the consequence of their presence at these concentrations is unknown. The overall potential for antibiotic removal by biological and physiochemical treatment systems and simultaneous risk of antibiotic resistance development has been relatively unexplored. The objective of this research was to investigate the effect of a representative pharmaceutical in a biological water reclamation system. The antibiotic evaluated in this study was amoxicillin, which is a semi-synthetic, beta-lactam antibiotic used for a variety of infections. The objective of this particular project is to determine the fate of amoxicillin in the City of Lubbock's Wastewater Reclamation Plant and determine the antibiotic resistance patterns in the plant.

Amoxicillin was detected in the influent of the LWRP on only one occasion during the eight-month study. Amoxicillin was not detected in the plant's flow streams (primary sludge or activated sludge or in the LWRP effluent). Due to the small percentage of the cities population on amoxicillin at any given time and the ease at which amoxicillin is degraded, it is unlikely that amoxicillin would be present in the influent of the LWRP at concentrations greater than detected in this study. In the LWRP, bacteria were resistant to multiple antibiotics. Resistance to beta-lactam antibiotics was common,

as indicated by the results of the and disk diffusion tests. The beta-lactam antibiotics investigated include penicillin, ampicillin, amoxicillin, and cephalothin. For the disk diffusion tests, amoxicillin was only available combined with the beta-lactamase inhibitor, clavulanic acid. In many cases, the beta-lactamase inhibitor was ineffective and organisms in the systems investigated were resistant to the beta-lactam, beta-lactamase inhibitor combination. Thus, the bacteria in the LWRP had the genetic mechanisms for beta-lactamase production, which provided resistance to beta-lactam antibiotics and beta-lactamase inhibiting compounds (i.e., clavulanic acid), which may be the consequence of overproduction of beta-lactamases or bacterial mutations in the clavulanic acid target.

The greatest concern is that antibiotic resistant bacteria in the effluent of the LWRP may spread the genetic information encoding antibiotic resistance to organisms in the environment of the LWRP outfall. As a consequence, changing the antibiotic resistance properties of the bacteria in the ecosystem may disrupt the ecosystem and the water may be a health hazard to end-users. For example, a person uses the LWRP water to irrigate their farmland. During an irrigation event, the farmer cuts his/her hand and the reclaimed wastewater comes into contact with the wound. Bacteria present in the water may infect the cut and the farmer may be hospitalized with a difficult infection to cure. This is a scenario is possible with the use of reclaimed wastewater.

One solution to the aforementioned situation is to thoroughly disinfect the wastewater before releasing to the environment or end-users. This will minimize the bacterial population in the wastewater and minimize the exposure of the receiving ecosystem to antibiotic resistant bacteria. However, disinfection requirements may need to become more stringent to protect the ecosystems downstream from the wastewater treatment plant's outfall.

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APPENDIX

Table A.1. LWRP Susceptibility test results for June.

INFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.2	0.7	1.0	1.0	0.3	Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	0.8	2.3	0.7	1.3	0.9	Susceptible
Rifampin	1.0	0.9	0.7	0.9	0.2	Resistant
Streptomycin	0.8	2.0	0.7	1.2	0.7	Mod. Resistant
Tetracycline	NA	NA	NA	NA	NA	NA
Vancomycin	0.8	0.7	0.7	0.7	0.1	Resistant
PRIMARY SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.3	1.1	1.0	1.1	0.2	Mod. Resistant
Ampicillin	0.7	0.9	0.7	0.8	0.1	Resistant
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.0	1.0	1.2	1.1	0.1	Mod. Resistant
Rifampin	0.9	1.2	0.9	1.0	0.2	Resistant
Streptomycin	1.0	1.2	1.0	1.1	0.1	Mod. Resistant
Tetracycline	NA	NA	NA	NA	NA	NA
Vancomycin	0.7	0.7	0.9	0.8	0.1	Resistant
ACTIVATED SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	2.8	1.1	1.4	1.8	0.9	Susceptible
Ampicillin	0.7	0.7	1.0	0.8	0.2	Resistant
Bacitracin	0.8	1.0	0.8	0.9	0.1	Resistant
Cephalothin	0.7	0.8	0.7	0.7	0.1	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.6	1.0	1.7	1.4	0.4	Susceptible
Rifampin	1.2	2.1	1.4	1.6	0.5	Susceptible
Streptomycin	1.4	1.2	2.4	1.7	0.6	Susceptible
Tetracycline	NA	NA	NA	NA	NA	NA
Vancomycin	0.8	0.8	0.7	0.8	0.1	Resistant
EFFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.8	2.4	1.4	1.9	0.5	Susceptible
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	0.7	0.7	1.1	0.8	0.2	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	2.1	1.9	1.9	2.0	0.1	Susceptible
Rifampin	1.2	1.2	1.1	1.2	0.1	Mod. Resistant
Streptomycin	1.9	2.2	2.2	2.1	0.2	Susceptible
Tetracycline	NA	NA	NA	NA	NA	NA
Vancomycin	0.8	0.8	0.9	0.8	0.1	Resistant

Table A.2. LWRP susceptibility test results for July.

INFLUENT	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.2	0.9	1.0	1.0	0.2	Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	0.7	0.7	0.7	0.7	0.0	Resistant
Rifampin	0.8	1.0	1.1	1.0	0.2	Resistant
Streptomycin	0.7	0.9	0.9	0.8	0.1	Resistant
Tetracycline	0.8	0.8	1.0	0.9	0.1	Resistant
Vancomycin	0.7	0.7	0.7	0.7	0.0	Resistant
PRIMARY SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.1	1.7	1.4	1.4	0.3	Susceptible
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	0.7	0.8	0.9	0.8	0.1	Resistant
Cephalothin	0.7	1.0	0.7	0.8	0.2	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.0	1.4	1.5	1.3	0.3	Susceptible
Rifampin	1.1	1.2	1.2	1.2	0.1	Mod. Resistant
Streptomycin	0.9	1.2	1.3	1.1	0.2	Mod. Resistant
Tetracycline	0.8	1.1	1.0	1.0	0.2	Resistant
Vancomycin	0.9	0.9	0.8	0.9	0.1	Resistant
ACTIVATED SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	0.7	1.1		0.9	0.3	Resistant
Ampicillin	0.7	0.7		0.7	0.0	Resistant
Bacitracin	0.7	0.8		0.8	0.1	Resistant
Cephalothin	0.7	0.7		0.7	0.0	Resistant
Penicillin	0.7			0.7		Resistant
Ciprofloxacin	0.7	0.7	0.8	0.7	0.1	Resistant
Rifampin	1.0	1.5	0.8	1.1	0.4	Mod. Resistant
Streptomycin	0.7	0.7	0.7	0.7	0.0	Resistant
Tetracycline	1.0	0.7	0.7	0.8	0.2	Resistant
Vancomycin	0.7	0.7	0.7	0.7	0.0	Resistant
EFFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.8	1.8	1.6	1.7	0.1	Susceptible
Ampicillin	0.8	0.7	0.7	0.7	0.1	Resistant
Bacitracin	0.8	0.9	0.8	0.8	0.1	Resistant
Cephalothin	0.8	1.0	1.0	0.9	0.1	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.1	0.8	0.7	0.9	0.2	Resistant
Rifampin	1.2	1.4	1.0	1.2	0.2	Mod. Resistant
Streptomycin	1.6	1.4	8.0	3.7	3.8	Susceptible
Tetracycline	1.6	1.4	1.2	1.4	0.2	Susceptible
Vancomycin	0.8	1.0	0.8	0.9	0.1	Resistant

Table A.3. LWRP susceptibility test results for August.

INFLUENT	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	0.7	0.8	1.0	0.8	0.2	Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	0.9	0.7	1.0	0.9	0.2	Resistant
Rifampin	1.1	0.7	1.4	1.1	0.4	Mod. Resistant
Streptomycin	1.4	1.1	1.1	1.2	0.2	Mod. Resistant
Tetracycline	0.8	1.0	1.0	0.9	0.1	Resistant
Vancomycin	0.7	0.7	0.8	0.7	0.1	Resistant
PRIMARY SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.1	1.0	1.3	1.1	0.2	Mod. Resistant
Ampicillin	0.8	0.7	0.7	0.7	0.1	Resistant
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.2	1.1	1.1	1.1	0.1	Mod. Resistant
Rifampin	1.0	1.2	1.0	1.1	0.1	Mod. Resistant
Streptomycin	1.0	1.4	1.4	1.3	0.2	Susceptible
Tetracycline	1.2	1.0	1.2	1.1	0.1	Mod. Resistant
Vancomycin	0.7	0.7	0.7	0.7	0.0	Resistant
ACTIVATED SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.8	1.7	1.2	1.6	0.3	Susceptible
Ampicillin	1.0	1.2	1.6	1.3	0.3	Susceptible
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.4	2.8	1.8	2.0	0.7	Susceptible
Rifampin	1.0	0.8	1.1	1.0	0.2	Mod. Resistant
Streptomycin	1.4	1.9	1.2	1.5	0.4	Susceptible
Tetracycline	1.0	1.6	1.8	1.5	0.4	Susceptible
Vancomycin	0.7	0.7	0.7	0.7	0.0	Resistant
EFFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.2	2.2	2.4	1.9	0.6	Susceptible
Ampicillin	1.0	1.2	1.0	1.1	0.1	Mod. Resistant
Bacitracin	0.8	1.0	0.7	0.8	0.2	Resistant
Cephalothin	0.7	0.7	2.2	1.2	0.9	Mod. Resistant
Penicillin	0.7	0.7	1.2	0.9	0.3	Resistant
Ciprofloxacin	1.0	2.0	2.4	1.8	0.7	Susceptible
Rifampin	1.8	1.6	2.2	1.9	0.3	Susceptible
Streptomycin	1.1	0.8	0.7	0.9	0.2	Resistant
Tetracycline	3.8	4.0	3.8	3.9	0.1	Susceptible
Vancomycin	1.2	1.0	1.8	1.3	0.4	Susceptible

Table A.4. LWRP susceptibility test results for September.

INFLUENT	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.0	1.1	1.4	1.2	0.2	Mod. Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	-	-	-	-	-	-
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.3	1.4	1.2	1.3	0.1	Susceptible
Rifampin	-	-	1.1	1.1	-	Mod. Resistant
Streptomycin	-	0.8	-	0.8	-	Resistant
Tetracycline	0.8	1.1	0.9	0.9	0.2	Resistant
Vancomycin	0.7	-	-	0.7	-	Resistant
PRIMARY SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.2	1.2	1.2	1.2	0.0	Mod. Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	-	-	-	-	-	-
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.3	1.4	1.2	1.3	0.1	Susceptible
Rifampin	1.0	-	-	1.0	-	Resistant
Streptomycin	-	1.0	-	1.0	-	Resistant
Tetracycline	1.0	1.0	1.0	1.0	0.0	Resistant
Vancomycin	-	-	0.7	0.7	-	Resistant
ACTIVATED SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	0.9	1.3	1.2	1.1	0.2	Moderately Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	-	-	-	-	-	-
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.1	1.6	1.6	1.4	0.3	Susceptible
Rifampin	1.5	-	-	1.5	-	Susceptible
Streptomycin	-	0.8	-	0.8	-	Resistant
Tetracycline	1.1	1.1	1.0	1.1	0.1	Mod. Resistant
Vancomycin	-	-	0.7	0.7	-	Resistant
EFFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.3	0.8	1.0	1.0	0.3	Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	-	-	-	-	-	-
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	0.7	0.8	0.8	0.8	0.1	Resistant
Rifampin	1.0	-	-	1.0	-	Resistant
Streptomycin	1.1	0.7	-	0.9	0.3	Resistant
Tetracycline	0.7	0.7	0.8	0.7	0.1	Resistant
Vancomycin	0.7	-	0.7	0.7	0.0	Resistant

Table A.5. LWRP susceptibility test results for October.

INFLUENT	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.2	1.5	1.4	1.4	-	Susceptible
Ampicillin	0.7	0.7	-	0.7	-	Resistant
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.9	0.7	-	0.8	-	Resistant
Penicillin	0.7	0.7	-	0.7	-	Resistant
Ciprofloxacin	0.7	0.7	-	0.7	-	Resistant
Rifampin	0.8	0.8	-	0.8	-	Resistant
Streptomycin	1.9	1.0	-	1.5	-	Susceptible
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant
PRIMARY SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	0.9	1.2	-	1.1	-	Mod. Resistant
Ampicillin	0.7	0.7	-	0.7	-	Resistant
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.7	0.7	-	0.7	-	Resistant
Penicillin	0.7	0.7	-	0.7	-	Resistant
Ciprofloxacin	1.4	0.9	-	1.2	-	Mod. Resistant
Rifampin	0.7	0.7	-	0.7	-	Resistant
Streptomycin	0.7	0.7	-	0.7	-	Resistant
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant
ACTIVATED SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.6	1.6	-	1.6	-	Susceptible
Ampicillin	0.7	0.7	-	0.7	-	Resistant
Bacitracin	0.7	0.7	-	-	-	Resistant
Cephalothin	0.7	0.7	-	0.7	-	Resistant
Penicillin	0.7	0.7	-	0.7	-	Resistant
Ciprofloxacin	0.9	0.7	-	0.8	-	Resistant
Rifampin	0.7	0.7	-	0.7	-	Resistant
Streptomycin	0.8	1.7	-	1.3	-	Susceptible
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant
EFFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.8	2.2	-	2.0	-	Susceptible
Ampicillin	1.2	1.4	-	1.3	-	Susceptible
Bacitracin	0.7	0.8	-	0.8	-	Resistant
Cephalothin	1.2	1.2	-	1.2	-	Mod. Resistant
Penicillin	1.2	0.9	-	1.1	-	Mod. Resistant
Ciprofloxacin	2.0	1.2	-	1.6	-	Susceptible
Rifampin	1.0	-	-	1.0	-	Resistant
Streptomycin	2.0	1.6	-	1.8	-	Susceptible
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant

Table A.6. LWRP susceptibility test results for November.

INFLUENT	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	0.7	1.9	2.0	1.5	0.7	Susceptible
Ampicillin	1.1	1.2	0.7	1.0	0.3	Resistant
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.8	0.8	0.7	0.8	0.1	Resistant
Ciprofloxacin	2.0	1.5	1.6	1.7	0.3	Susceptible
Rifampin	0.7	0.7	-	0.7	-	Resistant
Streptomycin	0.7	0.8	-	0.8	-	Resistant
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	-	-	0.7	-	Resistant

PRIMARY SLUDGE	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.1	0.8	0.8	0.9	0.2	Resistant
Ampicillin	0.7	0.7	0.8	0.7	0.1	Resistant
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.0	1.0	1.5	1.2	0.3	Mod. Resistant
Rifampin	0.8	0.8	-	0.8	-	Resistant
Streptomycin	1.0	0.7	-	0.9	-	Resistant
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant

ACTIVATED SLUDGE	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.6	1.6	2.0	1.7	0.2	Susceptible
Ampicillin	1.2	0.7	0.8	0.9	0.3	Resistant
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.4	2.0	1.2	1.5	0.4	Susceptible
Rifampin	0.7	1.0	-	0.9	-	Resistant
Streptomycin	0.7	1.4	-	1.1	-	Mod. Resistant
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant

EFFLUENT	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	2.0	2.2	1.1	1.8	0.6	Susceptible
Ampicillin	1.5	1.3	2.4	1.7	0.6	Susceptible
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.7	1.0	1.6	1.1	0.5	Mod. Resistant
Penicillin	0.7	1.2	0.7	0.9	0.3	Resistant
Ciprofloxacin	2.4	1.4	2.2	2.0	0.5	Susceptible
Rifampin	1.7	0.9	-	1.3	-	Susceptible
Streptomycin	1.6	0.9	-	1.3	-	Susceptible
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant

Table A.7. LWRP susceptibility test results for December.

INFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	0.9	0.9	0.9	0.9	0.0	Resistant
Ampicillin	0.7	0.7	0.8	0.7	0.1	Resistant
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.8	0.7	0.7	0.7	0.1	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.2	1.1	1.0	1.1	0.1	Mod. Resistant
Rifampin	0.7	0.7	0.8	0.7	0.1	Resistant
Streptomycin	0.7	0.7	0.8	0.7	0.1	Resistant
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	0.7	0.7	0.0	Resistant
PRIMARY SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	0.9	1.0	1.0	1.0	0.1	Resistant
Ampicillin	0.7	0.8	0.8	0.8	0.1	Resistant
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.5	1.5	1.1	1.4	0.2	Susceptible
Rifampin	1.1	1.1	-	1.1	-	Mod. Resistant
Streptomycin	0.8	0.8	-	0.8	-	Resistant
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant
ACTIVATED SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.0	1.6	1.4	1.3	0.3	Susceptible
Ampicillin	0.8	0.7	0.7	0.7	0.1	Resistant
Bacitracin	0.8	0.8	-	0.8	-	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.8	0.7	0.1	Resistant
Ciprofloxacin	1.2	1.2	1.2	1.2	0.0	Mod. Resistant
Rifampin	0.9	0.8	-	0.9	-	Resistant
Streptomycin	1.6	1.1	-	1.4	-	Susceptible
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant
EFFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	2.8	2.0	1.0	1.9	0.9	Susceptible
Ampicillin	1.0	1.4	2.0	1.5	0.5	Susceptible
Bacitracin	0.7	1.0	1.6	1.1	0.5	Resistant
Cephalothin	1.4	0.9	0.7	1.0	0.4	Resistant
Penicillin	2.0	1.0	0.7	1.2	0.7	Resistant
Ciprofloxacin	2.0	1.6	2.2	1.9	0.3	Susceptible
Rifampin	1.5	1.2	0.7	1.1	-	Mod. Resistant
Streptomycin	3.1	1.8	2.0	2.3	-	Susceptible
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	1.0	0.9	0.9	-	Resistant